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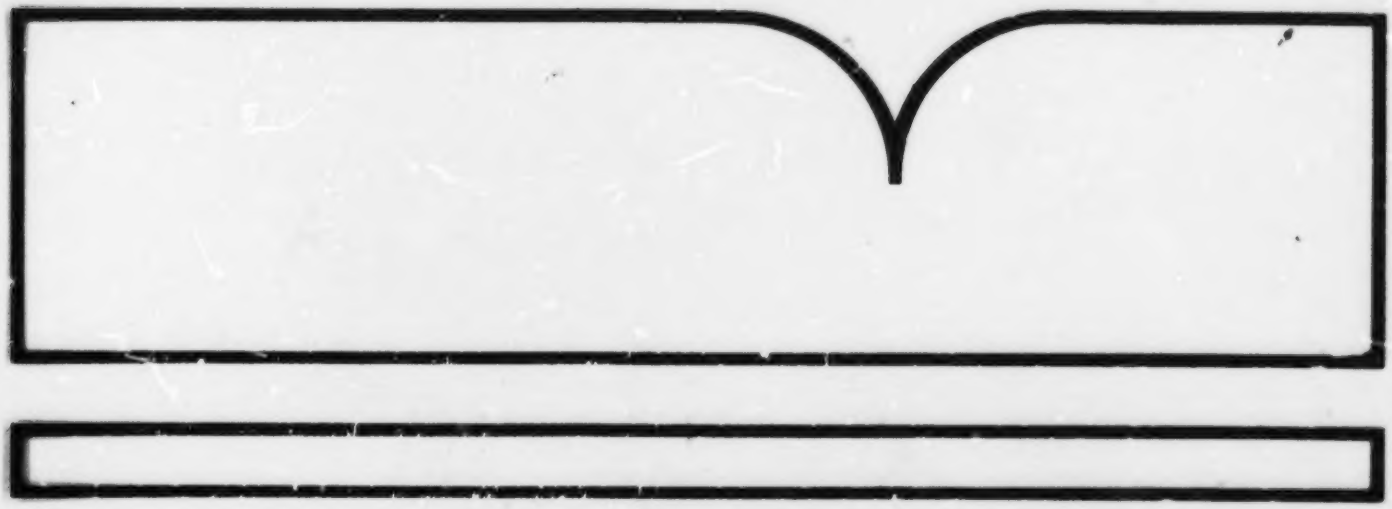

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DEVELOPMENT OF A TEST-TUBE STRESS-ETHYLENE BIOASSAY FOR DETECTING PHYTOTOXIC GASES*

L. E. CRAKER & J. J. FILLATTI

Department of Plant and Soil Sciences, University of Massachusetts, Amherst, Massachusetts 01003, USA

ABSTRACT

A rapid, quantitative bioassay for detecting phytotoxic air pollutants has been developed. The technique uses wheat Triticum aestivum *L. or tomato* Lycopersicon esculentum *L., seedlings growing on an agar medium in test-tubes. The seedlings are exposed to a pollutant in the test-tube and stress-ethylene induced by the pollutant is quantitatively measured by gas chromatography. Increases in ethylene production from seedlings exposed to a phytotoxic air pollutant as compared with controls not exposed to pollutants were related to the pollutant concentration.*

INTRODUCTION

The presence of a phytotoxicant in an air sample has generally been recognised through the development of tissue injury on plants exposed to the air sample (Heggestad, 1968; Hindawi, 1970; Jacobson & Hill, 1970; LaCasse & Treshow, 1976). However, since injury patterns induced by phytotoxic substances can vary from species to species and from pollutant to pollutant, assessments of air quality through observation of pollution-induced injury are often subject to human bias (Knudsen *et al.*, 1977).

Attempts have been made to use variables such as loss of leaf chlorophyll (Craker, 1971*b*; Knudsen *et al.*, 1977), reduced growth (Tingey *et al.*, 1971), altered development (Koukol & Dugger, 1967; Craker & Feder, 1972), reduced photosynthesis (Todd & Probst, 1963), light reflectance (Runeckles & Resh, 1975), and production of stress-ethylene (Tingey *et al.*, 1976, 1978) or stress-ethylene and ethane (Bressan *et al.*, 1979) as indicators of phytotoxic pollutants. All of these techniques, however, have involved a large commitment of time and facilities

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because of the relatively long-term growth period required for test plants plus the large quantities of pollutants and special fumigation chambers necessary for exposing plants to pollutants. The current procedure for utilising stress-ethylene production as a measure of phytotoxic activity requires growing bush beans to the 1·5 plastochron index, collecting approximately 1000 litres of pollutant and exposing the bean plants to the pollutant in large treatment chambers (Duke *et al.*, 1977; Brusick *et al.*, 1980).

This study reports on the development of a rapid, quantitative bioassay for detecting the phytotoxic activity in air samples using pollutant-induced stress-ethylene production from wheat or tomato seedlings growing on an agar medium in test-tubes.

MATERIALS AND METHODS

Wheat *Triticum aestivum* L. cv. Olaf, and tomato *Lycopersicon esculentum* L. cv. Roma VF, seedlings, growing in test-tubes were used in these studies. Wheat seeds, cold-stratified for 5 days at 5 °C, and tomato seeds, imbibed in distilled water at 25 °C for 24 h, were surface-sterilised in a 1 % sodium hypochlorite solution for 10 min, rinsed with distilled water and individually planted in 23 ml (1·5 × 15 cm) test-tubes containing 3 ml of sterilised agar growth medium (1 % agar and half-strength Hoagland's solution (Hoagland & Arnon, 1950)). Following planting, test-tubes containing seeds were stoppered with cotton plugs to prevent airborne pathogenic contamination and placed into controlled environment chambers for germination and growth (16 h day/8 h night cycle; 65 μE m^{-2} s^{-1} (400–700 nm), with a constant temperature of 18 °C for wheat, 27 °C for tomato). To provide for water loss and to maintain stomatal openings, 0·5 ml of half-strength Hoagland's solution was supplied to wheat plants every 5 days and to tomato plants the day before exposure to pollutants.

Exposure of plant tissue to air pollutants and collection of stress-ethylene was accomplished by replacing the cotton plugs of each test-tube with a rubber serum cap and injecting pollutant into the sealed tube with a syringe and needle to produce a known concentration. Each test-tube was then returned to the same controlled environment chamber used to grow the seedlings for an exposure/collection period during which the pollutant interacted with the seedlings and the stress-ethylene produced by the seedlings in response to the pollutant treatment was trapped in the sealed test-tube. At the end of the exposure/collection period, a 2 ml gas sample was removed from each test-tube and quantitatively analysed for ethylene by gas chromatography (Craker, 1971*a*). Following ethylene analysis, fresh weight of the top growth from each plant was determined.

Control seedlings were grown and the test-tubes sealed in the same manner as for pollutant-treated seedlings, except that there was no pollutant treatment to seedlings. Differences in ethylene production per unit fresh weight of leaf tissue by

pollutant-treated plants and control plants indicated the stress-ethylene resulting from pollutant exposure. The optimum age at which the seedlings could be effectively used to test for phytotoxicity was determined by exposing seedlings of different ages to pollutants for 2 h. The most effective length of the exposure/collection period was determined by monitoring ethylene production from seedlings exposed to pollutants for different lengths of time.

All tests contained a minimum for five samples per treatment and were replicated a minimum of three times.

RESULTS

Measurable increases in ethylene production were observed in both wheat (Table 1) and tomato (Table 2) seedlings exposed to phytotoxic air pollutants. The amount of ethylene produced varied with both the pollutant and the plant species. Wheat plants treated with 200 μl litre^{-1} of carbon monoxide (a non-phytotoxic gas except at very high concentrations (LaCasse & Treshow, 1978)) did not produce ethylene above that of control plants.

The effect of age on sensitivity of seedlings to phytotoxic air pollutants is

TABLE 1

STRESS-ETHYLENE PRODUCTION BY WHEAT SEEDLINGS EXPOSED TO PHYTOTOXIC AIR POLLUTANTS. THE WHEAT SEEDLINGS WERE GROWN ON AN AGAR MEDIUM IN TEST-TUBES FOR 6 DAYS AND THEN EXPOSED TO THE INDICATED CONCENTRATIONS OF POLLUTANTS FOR 2h. MEAN ± SE.

Pollutant	*Ethylene production* nl g^{-1} *fresh weight 2 h*	*% above control*
Cl_2 at 100 μl litre^{-1}	4·12 ± 0·25	182
SO_2 at 100 μl litre^{-1}	2·15 ± 0·18	47
NO_2 at 100 μl litre^{-1}	2·05 ± 0·14	40
H_2S at 100 μl litre^{-1}	1·56 ± 0·31	7
CO at 200 μl litre^{-1}	1·38 ± 0·12	0
Control	1·46 ± 0·16	0

TABLE 2

STRESS-ETHYLENE PRODUCTION BY TOMATO SEEDLINGS EXPOSED TO PHYTOTOXIC AIR POLLUTANTS. THE TOMATO SEEDLINGS WERE GROWN ON AN AGAR MEDIUM IN TEST-TUBES FOR 11 DAYS AND THEN EXPOSED TO INDICATED CONCENTRATIONS OF POLLUTANTS FOR 2 h. MEANS ± SE.

Pollutant	*Ethylene production* nl g^{-1} *fresh weight 2 h*	*% above control*
Cl_2 at 100 μl litre^{-1}	4·49 ± 0·07	193
SO_2 at 100 μl litre^{-1}	2·55 ± 0·47	67
NO_2 at 400 μl litre^{-1}	2·43 ± 0·17	59
H_2S at 400 μl litre^{-1}	2·81 ± 0·50	84
Control	1·53 ± 0·21	0

illustrated in Fig. 1 using data from tomato seedlings of different ages exposed to Cl_2. Significant differences in ethylene production between seedlings exposed to phytotoxic air pollutants and controls were observed in wheat at all seedling ages tested and in tomato at all seedling ages tested except for 5 days. The level of ethylene production, however, was related to growth and development, with seedlings younger than 5 days in wheat and 9 days in tomato having relatively high rates of ethylene production compared with older seedlings. Following this initial high rate, ethylene production remained constant over the range of seedling ages tested.

Measurements of ethylene production over different exposure/collection periods are shown in Fig. 2 for tomato seedlings treated with chlorine. A similar response was observed in chlorine-treated wheat. Significant differences between pollutant-treated and control plants were evident after a 1 h exposure/collection period. After 2 h, the rate of ethylene production was reduced in both treated and control plants. Extending the exposure/collection period beyond 2 h did not substantially increase the differences in ethylene production between treated and control plants.

The relationship between stress-ethylene production and pollutant concentration for Cl_2, SO_2, NO_2 and H_2S is summarised in Table 3. For all the phytotoxic gases tested, there was a range of pollutant concentrations where an increase in concentration initiated a linear increase in ethylene production. Concentrations above this range gave only small—or no—further increases in production of stress-ethylene by the plant tissue, depending upon the pollutant. In tomato seedlings treated with sulphur dioxide, the minimum threshold for inducing stress-ethylene production was above 20 μl litre^{-1}.

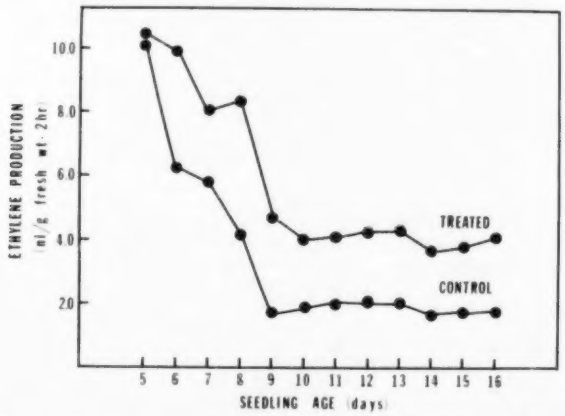

Fig. 1. Seedling age and stress-ethylene production. Tomato seedlings, grown on an agar medium in test-tubes for indicated days, were either exposed to 100 μl litre^{-1} Cl_2 for 2 h (Treated) or not treated with a pollutant (Control).

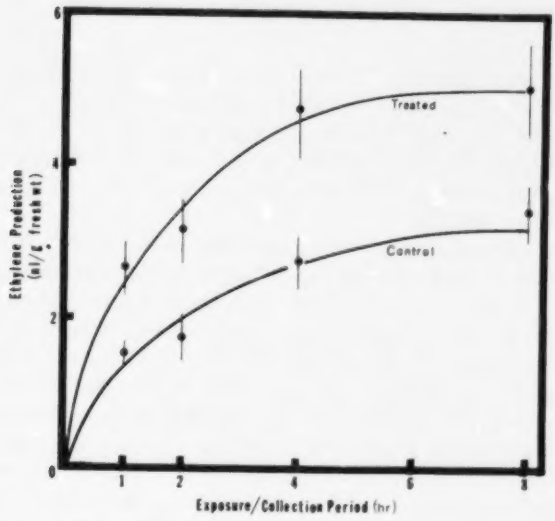

Fig. 2. Time course of stress-ethylene production. Tomato seedlings, grown on an agar medium in test-tubes for 11 days, were either exposed to 40 μl litre^{-1} Cl_2 for indicated times (Treated) or not treated with a pollutant (Control). Vertical lines indicate SE.

TABLE 3

RELATIONSHIP OF ETHYLENE PRODUCTION TO POLLUTANT CONCENTRATION. SEEDLINGS GROWING ON AN AGAR MEDIUM WERE EXPOSED TO THE INDICATED CONCENTRATIONS OF AIR POLLUTANTS FOR 2 h

Plant species	*Pollutant and range tested* (μl litre^{-1})	*Linear response range* (μl litre^{-1})	*Regression equation for linear responses*[a]	r^2[b]
Wheat	Cl_2, 0–100[c]	0–80	$Y = 2{\cdot}00 + 0{\cdot}44X$	0·90
	SO_2, 0–100[c]	0–100	$Y = 1{\cdot}09 + 0{\cdot}013X$	0·90
	NO_2, 0–400[d]	0–400	$Y = 1{\cdot}64 + 0{\cdot}003X$	0·90
	H_2S, 0–400[d]	0–400	$Y = 1{\cdot}41 + 0{\cdot}004X$	0·90
Tomato	Cl_2, 0–100[c]	0–40	$Y = 1{\cdot}86 + 0{\cdot}045X$	0·90
	SO_2, 0–100[c]	20–100	$Y = 0{\cdot}68 + 0{\cdot}022X$	0·94
	NO_2, 0–400[d]	0–400	$Y = 1{\cdot}21 + 0{\cdot}002X$	0·98
	H_2S, 0–400[d]	0–300	$Y = 1{\cdot}73 + 0{\cdot}004X$	0·79

[a] All regression coefficients were significant at $p \leq 0{\cdot}001$.
[b] Coefficient of determination.
[c] Concentrations of pollutant increased in units of 20 μl litre^{-1}.
[d] Concentrations of pollutant increased in units of 100 μl litre^{-1}.

DISCUSSION

The technique developed in this study is similar to others where stress-ethylene produced by plants treated with gaseous pollutants (Tingey *et al.*, 1976, 1977; Bressan *et al.*, 1979) has been used to detect phytotoxic air pollutants except that, in this instance, young seedlings growing in test-tubes were used as the test plant material and the test-tubes served as both the exposure chamber and container for collection of stress-ethylene.

The constancy of ethylene production by wheat seedlings ranging in age from 5 to 16 days from seeding and by tomato seedlings ranging in age from 9 to 16 days from seeding suggested that any age seedling within these ranges could be used in a stress-ethylene test for phytotoxicants. However, there were limitations created by the size of the test-tube. After 8 days in wheat and 12 days in tomato, the seedlings had grown so large that there was a physical restriction on continued leaf expansion in tomato due to the sides of the tube and in wheat due to the cotton stopper at the top of the tube. In the case of wheat over 8 days old, the tissue was easily injured when the rubber serum cap was placed in the top of the test-tube. To avoid problems of increased ethylene production due to growing and mechanical stresses (Abeles, 1973), wheat plants less than 8 days old and tomato plants less than 12 days old should be used in the test procedure.

The relatively large amounts of ethylene produced in both the pollutant-treated and control seedlings at early ages (3 to 4 days in wheat; 5 to 8 days in tomato) were associated with the early development of the plant tissue (Abeles, 1973). To ensure that all seedlings have reached the stage of development where ethylene is produced at a constant level, seedlings older than 5 days in wheat and 9 days in tomato should be used. We recommend using 6-day-old wheat and 11-day-old tomato seedlings.

Our results indicated that an exposure/collection period of 2 h was adequate for both inducing the plant tissue to produce ethylene and for the collection of sufficient ethylene for measurement by gas chromatography. At exposure/collection periods less than 2 h, low concentrations of phytotoxic gases did not induce the production of enough stress-ethylene to permit a distinction to be made between control and pollutant-treated seedlings. Any collection period with a duration of 2 to 8 h (longest exposure/collection period studied) would be satisfactory, since the magnitude of difference in ethylene production between treated and control seedlings was maintained. However, the 2-h exposure/collection period was sufficient and allowed for completion of the phytotoxicity test within one working day.

A 2-h exposure/collection period for pollutant-induced stress-ethylene is consistent with Saltveit & Dilley's (1978) characterisation of ethylene response to physical wounding as a rapidly induced transitory period of ethylene production. The decline in rate of ethylene production by both pollutant-treated and control plants associated with longer exposure/collection periods is undoubtedly caused by

environmental limitations of the sealed test-tube preventing continuation of plant metabolic processes.

The amount of ethylene produced by pollutant-treated wheat and tomato seedlings was related to the concentration of the pollutant to which the plants were exposed. With each pollutant, ethylene production increased as the concentration of the pollutant was increased. This relationship between ethylene production and pollutant concentration is similar to the results of other investigations where exposure to increased pollutant concentrations increased ethylene production (Craker, 1971*a*; Bressan *et al.*, 1979; Tingey *et al.*, 1976, 1978). The linear response between pollutant concentration and stress-ethylene production observed in these studies indicates that the bioassay could be used to determine the levels of phytotoxicant within an air sample.

The development of the bioassay has minimised the quantity of test gas necessary for a phytotoxicity test and reduced the need for glasshouse fumigation facilities. However, use of the bioassay is currently limited to air samples containing high concentrations of pollutants. Production of stress-ethylene from wheat or tomato seedlings required treatment with concentrations of SO_2, Cl_2, NO_2 and H_2S much higher than those normally shown to injure plants in the ambient environment (LaCasse & Treshow, 1976). Although the exact reason for the low sensitivity of the bioassay is unknown, an important factor may be the dissolution of the pollutants into the agar growth media. For example, under the sealed test-tube, static environmental conditions of the bioassay (water:air volume ratio of 3:19) and a Henry's law constant of approximately 0·0332 (Johnstone & Leppla, 1934; Hales & Sutter, 1973), much of the SO_2 would be in the condensed phase at equilibrium. Attainment of equilibrium is probably very rapid, at most a few minutes, and limited only by diffusion. Thus any SO_2 added to the test-tube for treating the plants would be unavailable for inducing stress-ethylene after only a short period of time.

The bioassay outlined in this paper should be useful to government and industrial laboratories in phytotoxic activity screening of smokestack emissions, industrial compounds and other air samples containing high pollutant concentrations. The procedure can be replicated a large number of times in a matter of hours, uses a defined test environment, requires a minimum amount of space and provides an unbiased estimate of phytotoxic activity. The bioassay has given a positive response to SO_2 in tests conducted over 18 months.

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